A High-Yield Sampler for Toxicological Characterization of Complex Mixtures in Combustion Effluents

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Combustion sampling for toxicological assessment often requires that large (>100 mg) lots of complex organic mixtures of wide volatility range be rapidly recovered from high temperature gases without contamination. A new sampler, meeting these criteria for studies of public health interest, has been developed and demonstrated. The device provides high sampling rates and intimate contacting of the sampled stream with large volumes of a well-cooled, liquid solvent, dichloromethane (DCM). This promotes rapid organics dissolution from carrier gas and particulates and prompt dilution and quenching of the resulting solution, resulting in high organics collection efficiencies with minimal DCM losses. Solvent separation then remits large quantities of concentrated organics for chemical analysis and toxicological testing. One- to seven-hour interrogations of in-flame, post-flame, and flue gas regions gave 50- to 250-mg yields of complex organic mixtures. In side-by-side sampling of combustion exhaust, the DCM sampler provided higher yields of DCM solubles (identified with complex organic mixtures) and of S. typhimuirim mutagens (active without exogenous metabolizing agents) than did a filter/polymeric sorbent bed sampling train. The new sampler also collects polar and high volatile hydrocarbons such as benzaheyde, pentadiyne, m- and p-diethynyl-benzene, and 1-hexen-3,5-diyne. Nitration of naphthalene and pyrene in DCM solution (1 mg/mL each) was less than 1 part in 10⁷ after a 345-min exposure to a bubbling flow of moist N₂/air mixture (1:1 v/v) containing 107 ppm NO and 1.5 ppm NO₂, indicating that for these condition a DCM sampler should resist artifactual nitration of aromatics. However, because of the very high bacterial mutagenicity of some nitroaromatics and the wide range of sampling conditions of environmental interest, nitration and all artifacts must still be scrutinized when using the DCM sampler. The DCM sampler is expected to contribute to public health impact assessments by facilitating detailed determinations of the identities, compositions, concentrations, sources, formation mechanisms, and biological activity of environmental toxicants in gaseous atmospheres.

Introduction

Combustion systems emit complex organic mixtures showing toxicological activity in bacterial cells (1-3), human cells (4,5), and rodents (W. F. Busby, Jr., personal communication). Progress in emissions monitoring and control has been encouraging. However, combustion will remain of interest as an important potential source of environmental toxicants because of continued need for fossil and biomass-fueled stationary and mobile com-

bustors and for incineration of municipal refuse and hazardous wastes. In-flame and exhaust region sampling are important for quantifying ambient concentrations, chemical composition, and bioactivity of combustion emissions, contributing critical data for determining toxicants emissions factors, sources, and formation mechanisms, and for devising control strategies for combustion-derived toxicants. Most characterization measurements are performed off line. Reliable toxicological assessments can easily require that > 100 mg samples of complex organic mixtures of wide volatility range be expeditiously recovered from high temperature gases without contamination. For instance, assay for lung adenoma induction in the CD-1 strain of newborn mice requires up to 100 mg of test material for dose-response measurements in appropriate numbers of animals (6). Human cell mutagenicity assays require testing at doses of up to 0.1 mg/mL and up to 100 mL of test solution, corresponding to a total sample requirement of 10 mg. Detailed chemical analyses to identify specific chemicals responsible for toxicological activity can consume a further 100 to 200 mg of mixture.

State-of-the-art methods for combustion sampling of complex

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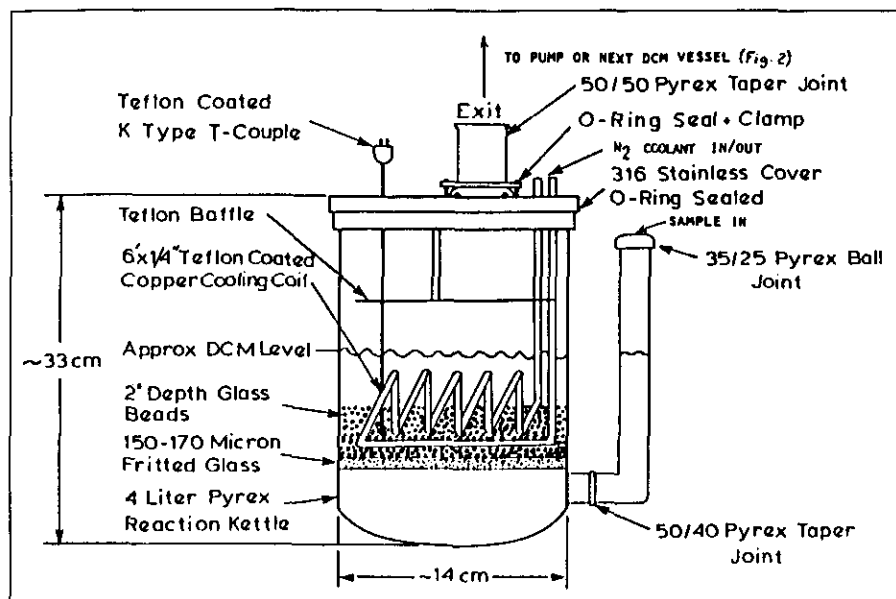
FIGURE 1. Cooled DCM contacting vessel.

organic mixtures typically divert a known fraction of effluent through an invasive probe and collect polycyclics and other organic compounds on filters and within one or more postfilter traps, typically consisting of packed beds of polymeric sorbent particles. Organics are recovered for analysis by extracting each collection station with a strong, volatile organic solvent such as dichloromethane (DCM). Filter/sorbent bed sampling trains have exhibited several drawbacks in collecting complex organic mixtures for toxicological characterization. Sample distribution between filter and sorbent can be quite arbitrary, reflecting apparatus-specific effects of sampling rate, sample composition, and collection station temperature. Filters contribute to mutagenic artifacts formation (7-11) by concentrating higher molecular weight organics and promoting their reaction with NO$_x$ and other effluent gases. Potential problems with some sorbents include high organics backgrounds; loss of collection efficiency with extended sampling; sorbent atrophy and fragility; diminished performance under elevated temperatures; labor-intensive preparation and sample workup; and inadequate quantitative understanding or organics collection efficiencies under actual combustion sampling conditions.

This paper reports on the design, operation, and validation of an improved combustion sampler that furnishes high yields of complex organic effluents in reasonable collection times, while eliminating or significantly remediating the above deficiencies.

Design Strategy

The approach was to design a direct impingement collection vessel that at high sampling rates promoted intimate contacting between sampled gas and large volumes of a refrigerated, liquid solvent; rapid organics dissolution from sampled gas and particulates; prompt dilution and quenching of the resulting solution; and minimal solvent losses. DCM was chosen as the solvent because of its low freezing point, low flammability, high dissolution power for polycyclic and other organic compounds, and its

high volatility to facilitate sample recovery.

Safety note: DCM is a toxicant and is hazardous. Proper safety precautions must be followed in storage, handling, use, and disposal of this compound.

One motivation for the sampler design is to inhibit artifactual reactions by preserving the catch in cold, dilute solution throughout sampling, and by preventing sample concentration by filters or sorbent surfaces. Simplicity is a further motivation, since this design requires only one operation, solvent separation, for sample recovery. The present approach is somewhat reminiscent of various sampling trains where the freshly diverted sampling stream was directed through one or more impinger vessels. For example, almost three decades ago Stenberg et al. (12) published on their sampling of automotive and incinerator emissions for benzo(a)pyrene by educting exhaust through cooled water bubblers and then through a particulate filter.

Sampler Description

The sampling train consists of two or more series-connected DCM contacting vessels (impingers) (Fig. 1), to collect, cool, dilute, and preserve the catch. Each vessel is a 0.45 cm wall, 4-L cylindrical pyrex reaction kettle (~ 30 cm deep, length to diameter of about 2.4), divided into inlet (~ 1 L) and main (~ 3 L) chambers by a 0.64-cm thick fritted glass disk with 150 to 170 μm orifices. A 5-cm deep layer of no. 4 or 6 glass beads is placed above the frits in impingers 1 and 2 to aid gas-liquid contacting and to break up aerosols formed by rapid cooling of the sampled stream.

The inlet chamber precools the sampled stream and separates residual moisture as ice in the first impinger. The fritted disk causes the sampled stream to enter the main chamber as a torrent of tiny bubbles carrying sufficient momentum to stir the solvent. Bubbling, stirring, and the use of a large solvent volume combine to promote organics dissolution from the carrier gas and entrained particulates by increasing gas-liquid contact areas, by providing a high concentration driving force for dissolution (by
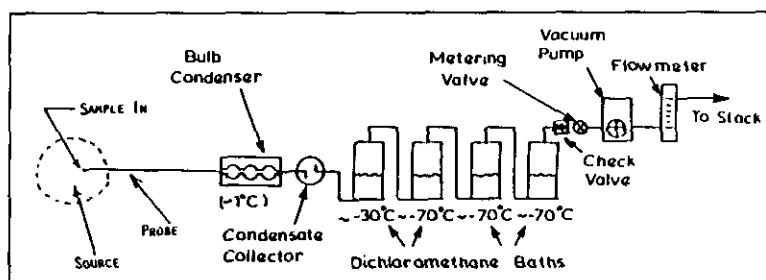
FIGURE 2.   Typical DCM sampling train.

diluting and homogenizing the resulting solution of sample), and by reducing interphase transport lengths (by converting the sampled gas to bubbles). These design and operating features provide good sample collection efficiencies with a manageable size contacting vessel while cooling and diluting the sample to resist artifactual reactions, even for long collection times.

The solvent temperature in the main chamber of the contacting vessel (Fig. 1) is monitored by a Teflon-coated type-K thermocouple and is independently regulated to within $\pm 2\,^\circ C$ by electronically controlling delivery of cold N_2 (from a liquid nitrogen tank) to a submerged heat exchange coil ($\sim$ 1.83 m of 0.64-cm OD Teflon-coated copper tubing) using a feedback signal from the thermocouple. A baffle (a 11.4-cm OD, 0.16-cm thick Teflon sheet) in the upper third of the main chamber inhibits solvent losses caused by entrainment in the escaping carrier gas. Teflon components are used to prevent sample contamination from metallic ions leachable from copper and other metals by acidic gases (CO_2, NO_2, and SO_2) in combustion effluents. During sampling, the first DCM reservoir is maintained at -30 $\pm$ 2 $^\circ C$ to prevent plugging of its frit by ice. This temperature reflects compromises between preventing frit icing and minimizing solvent and sample losses at high gathering rates. The remaining DCM reservoirs are typically operated at -70 $\pm$ 2 $^\circ C$ to increase organics solubility and to resist artifactual reactions and solvent losses while preventing DCM solidification. These temperatures were selected based on operating experience and depend on the solvent, sampling conditions, and the collection vessel geometry.

In the present applications, a typical sampling train (Fig. 2) consisted of an invasive probe to interrogate the medium of interest (materials, length, and internal diameter, here pyrex, $\sim$ 91 cm, and $\sim$ 2.54 cm, respectively, are chosen to be compatible with the temperature of the sampled medium, and to provide a well-defined sampling zone, convenient interfacing to downstream equipment, and desired sampling rates); a 65-cm long bulb condenser flushed with water thermostatted at $\sim$ 1$^\circ C$ to precool and partially dehumidify the sampled stream; a condensate collector; two or more series-connected DCM contacting vessels to collect, dilute, and preserve the sample; a check valve to prevent backstreaming of educted effluent or atmospheric gases to the collector vessels; a manual metering valve to select the sampling rate; a vacuum pump; and a flow meter to measure the sampling rate. With proper attention to interfacing and sample acquisition design procedures, the DCM sampler should be compatible with other probes.

Operating Procedure

Before sampling, each collection vessel is charged with about 2 L of DCM. The DCM reservoirs and the condenser are then cooled to their desired operating temperatures by directing the appropriate coolants (N_2 or water, respectively) through the heat exchange coils or water jacket. During sampling, these temperatures are automatically regulated at the operating values specified above. The sampling rate is then set by activating the vacuum pump and adjusting the metering valve while monitoring the flowmeter (Fig. 2). Sampling then proceeds for the desired collection time by aspirating gas through the probe and DCM impingers, and, if necessary, occasionally adjusting the metering valve to keep the sampling rate within prescribed limits. Upon completing sampling, the DCM contacting vessels are disconnected from each other. The entrance and exit ports of each vessel are then lightly covered with aluminum foil, and each vessel is allowed to attain ambient temperature by natural warming.

Safety note: This warming causes dramatic increases in the DCM vapor pressure in each contacting vessel. Disconnecting the DCM vessels and then lightly covering the entrance and exit port of each with aluminum foil allows each DCM reservoir to separately equilibrate with the ambient atmosphere, enabling these pressure increases to be relieved as they develop. If these precautions are not followed, there is serious danger that the accumulating DCM vapor will overpressurize the vessel to the point of shattering, posing serious threat of personal injury. Thus, the DCM contacting vessels must never be tightly sealed before the DCM attains ambient temperature and before the pressure in the head space above the DCM liquid has become equilibrated with atmospheric pressure. Disconnecting the DCM vessels also prevents intermixing of vessel contents in case of more rapid warming and pressurization of individual DCM reservoirs.

After the DCM has reached ambient temperature, liquid water is separated from the condensate collector and from the first DCM vessel and extracted with DCM in a separatory funnel. The resulting DCM is then combined with the DCM from the first collector. The probe is rinsed with DCM, and the rinse is added to the first DCM collection vessel. The DCM from each of the collector vessels is then separately concentrated by Kuderna Danish (KD) evaporation. Typically, 6 hr are required for concentrating 2 L of DCM to 15 mL, using a six-ball Snyder column with a nominal 1.5 L KD concentrator, heated in a hot water bath at $\sim$ 80$^\circ C$. The resulting organic concentrates are then further worked up and subjected to chemical analysis and toxicological testing as desired.

Performance Demonstrations

Solvent Retention and Collection Efficiency for Reference Polyaromatic Hydrocarbon Compound

Two sampling tests were performed using two DCM collector

Table 1. Retention of reference aromatic compounds in first DCM impinger vessel.

Sampling or soak time, min	Sampling rate, SCFM[b]	% Initial charge recovered from bath 1 after sampling[a]			
		Bromobenzene	1-Bromonaphthalene	9-Bromophenanthrene	1-Bromopyrene
62	0.94	73	70	87	70
60	1.61	72	74	95	69
60	0[c]	92	80	86	84
60	0[c]	108	91	92	90

[a]About 200 mg of each compound was dissolved in 2 L of DCM in bath 1 prior to sampling live oil burner exhaust. Oil burner and typical operating procedures are described by Leary et al. (1). Temperatures: bath 1 ~ -30°C; bath 2 ~ -70°C.

[b]SCFM, standard cubic feet per minute. Continuous firing of No. 2 distillate fuel oil at low apparent smoke density (Bacharach Smoke No. 1).

[c]Control run, no flow.

vessels connected in series. In each experiment, 500 mg of pyrene were placed within the probe (Fig. 2) near its entrance, and residential oil burner exhaust [see Leary et al. (1) for burner description] was drawn through the probe and DCM collectors for about 1 hr. Collector 1 (~ −30°C) exhibited DCM losses of 5 and 15 volume % at sampling rates of 0.94 and 1.61 standard cubic feet per minute (SCFM), respectively, and collected 70% of the pyrene in each run.

Collection Reservoir Retention Efficiencies for Aromatic Compounds of Different Volatility

The two experiments just described were also used to assess the retention of individual reference polyaromatic hydrocarbon (PAH) compounds in the DCM impinger vessels. To this end, 200 mg each of bromobenzene, 1-bromonaphthalene, 9-bromo-phenanthrene, and 1-bromopyrene were dissolved in the first collection reservoir before sampling began. Table 1 shows that, generally, about 70 to > 90% of each compound was retained in Collector 1. Collector 2 typically contained ≤ 1% of each compound. Control (no flow) runs (Table 1) generally gave recovery efficiencies between about 80 and 90%, implying that about 10 to 20% of the initial charge of each compound is lost during sample concentration and analysis (here by gas chromatography) and not by escape from collector 1 during sampling. Thus, the true DCM reservoir retention efficiencies are about 80 to 90% for aromatics representing a broad range of volatility (boiling points of 156 to > 360°C).

Extent of Artifactual Nitration of Selected Aromatic Compounds

Nitrogen oxides, especially in the presence of water vapor and oxygen, are highly reactive and have been observed to cause nitration of organic compounds. Nitrogen-containing compounds, typically nitroarenes, can be formed under a remarkably wide range of conditions. Furthermore, nitration of organics has been shown to occur in gases (13–15), liquid solution (16), and on solid sorbents (7,17). Thus, artifact formation is a potential problem with any sampler operating in an NO_x environment and in the presence of oxygen and moisture.

To determine, for a set of conditions pertinent to combustion sampling, the possible extent of PAH nitration in the DCM sampler by NO_x-containing gases, 200 standard mL/min of a moist (bubbled through organics-free water) N_2/air mixture (1:1 v/v) containing 107 ppm NO and 1.5 ppm NO_2 was bubbled through 500 mL of a DCM solution of naphthalene and pyrene (1.0 mg/mL each) at ambient temperature for 345 min. The contacting vessel was a 1.5-L gas washing bottle (Lurex, Inc.) with a frit configuration similar to the DCM sampler in Figure 1 and

fitted with a reflux condenser at its exit to reduce solvent loss. After 345 min, the DCM solution of pyrene and naphthalene was concentrated from 500 to 5.0 mL in a KD concentrator and analyzed by gas chromatography with NO_2 selective pyrolysis/chemiluminescent detection (18–21). No nitroarenes were detected. Given the detection limit of the instrument (0.01 ng/μL injected for 1-nitropyrene) and the concentration factor of 100:1, nitration of the arene surrogates would be less than 1 part in 10^7. A control run without naphthalene or pyrene in the contacting vessel showed no nitration of the DCM after about 420 min of exposure but did reveal low levels of nitrogen-containing compounds, presumably generated by nitration of the DCM stabilizing agent (cyclohexene) or by nitration of DCM impurities.

Obviously, the formation of nitroarenes at levels below the detection limits of our analytical instruments (GC/ND, GC/MS, GC/FTIR) cannot be ruled out. This fact has important consequences in environmental sampling for toxicological assessment since some nitroarenes exhibit especially potent genotoxic action in mutagenicity assays based on S. typhimurium (22,23). Even at very low concentration levels of 0.1 ng/mL or less (based on the present findings of nitration of < 1 part in 10^7 of arenes present at 1 mg/mL), it is still possible that artifactually nitrated PAH could cause misleading mutagenicity determinations. Furthermore, wide ranges of operating conditions can be of interest in environmental sampling. Thus, nitration and all artifacts must still be scrutinized when employing the DCM sampler. For nitration artifacts, one practical approach is to spike the DCM reservoirs with nitration detectors, e.g., organic compounds known to be absent from the medium being sampled, but of comparable nitration reactivity to compounds expected in the sample. For example, in combustion sampling, a fully deuterated aromatic compound such as naphthalene-d_8 could be employed. The extent of artifactual nitration would then be measured by mass spectrometric determination of the yield of deuterated nitronaphthalene, which could only be generated by reactions within the DCM sampling train. For reliable measurements, the artifact formation behavior of a given sampler must always be established for the sampling conditions of interest.

Collection Efficiencies for Combustion Effluents

Table 2 shows the recoveries of DCM solubles (identifed with organic complex mixtures) sampled from a turbulent premixed ethylene/air flame (T ~ 1300°C) in a well-stirred combustor (WSR) described by Nenniger (24); and exhaust of a residential oil burner fired under cyclic, generally low-smoke emission conditions, of the type described by Leary et al. (1). In sampling the WSR at up to one-third SCFM, only a small percentage of the total recovered DCM solubles was detected in the DCM vessels downstream of the first collector, implying minimal sample

Table 2. Recovery of extractables from raw combustion effluents by successive DCM baths.

Combustor	Average sampling rate, SCFM	% Total sample in DCM bath no. [a]				Calculated extract yield, mg/kg fuel fired [d]
		1 [b]	2	3	4 [c]	
Residential oil burner, 66% excess air [e]	0.95	8.5	60.4	31.1	— [f]	888
Well-stirred combustor, ethylene at $\phi_f \geq 2.37$	0.16	94.7	3.3	2.0	— [f]	811
Residential oil burner, 62% excess air [e]	0.34	59.6	20.4	14.6	5.4	1304
Residential oil burner, 62% excess air [e]	0.42	13.3	39.5	18.5	28.7	974
Well-stirred combustor, ethylene at $\phi_f = 2.37$	0.36	94.7	1.6	0.8	2.9	1238

[a] Approximate bath temperatures: 1, -30°C; 2 through 4 (if used) -70°C.

[b] Includes DCM extract of water from knockout vessel.

[c] Glass beads were added to baths 1 and 2 in the 4-bath runs.

[d] DCM extractables.

[e] Nominal value. Other operating conditions as in Table 3.

[f] Bath 4 not used.

breakthrough and hence high recovery efficiencies at these gathering rates. The oil burner exhaust samples showed significant variability. Minimal product breakthrough (< 6%) was observed in a four collector sampling run at about one-third SCFM. However, in another oil burner experiment at similar nominal excess air, but somewhat higher sampling rates (~0.4 SCFM), average yields of DCM solubles were lower (974 vs. 1304 mg/kg fuel fired). Further, almost 30% of the total sample appeared in the farthest downstream collector (no. 4), implying that significantly more sample would have been obtained with additional downstream collectors. For somewhat larger nominal excess air, but a significantly higher sampling rate of 0.95 SCFM, yet another oil burner experiment showed almost one-third of the DCM solubles appearing in collector 3. The results of Table 2 suggest that for DCM collectors of the present size (Fig. 1), sampling rates above about one-third SCFM are to be avoided when quantitative data on organics yields, compositions, and total bioactivity are required.

Comparison of Extract Yields in Side-by-Side Sampling with the DCM Sampler and a Filter/Polymeric Sorbent Bed Sampling Train

Table 3 presents mean emitted yields of DCM extractables (solubles) measured by simultaneously sampling the flue gas from generally low smoke density cyclic firing (5 min on, 10 min off) of a residential oil burner with the DCM sampler and with a filter/polymeric sorbent bed sampler. Each sampler was connected to one arm of an inverted y-shaped probe. With two or four series-connected collection vessels, the DCM sampler gave roughly four times higher extract yields. The filter/sorbent bed results at 0.89 and 1.5 SCFM (Table 3) agree well with earlier studies of effluents from this oil burner at similar firing conditions by Leary et al. (1). Using a similar filter/sorbent bed sampling train and sampling rates of 5.5 to 9 SCFM, Leary et al. found emitted yields of DCM extractables to range from 24 to 69, with a mean of 34 mg/kg fuel fired (1). The multi-bath DCM sampler clearly provides superior product recovery efficiencies at similar sampling rates.

Comparison of Mutagens Emissions Estimated from Side-by-Side Sampling with the DCM and Filter/Sorbent Bed Samplers

Separate catches of DCM extractables were collected by simultaneously sampling flue gas from cyclic (5 min on, 10 min

off), generally low-smoke emissions firing of a residential oil burner using a four-vessel DCM sampling train, and a filter/polymeric sorbent bed sampler. Each sampling train was connected to one arm of an inverted Y-shaped probe. The products from each DCM bath and from combining the extracts of the filter, sorbent, and condensate were assayed for mutagenic activity to *S. typhimurium* without exogenous metabolizing agents, using the protocol of Skopek et al. (25,26).

This assay measures the fraction of bacterial cells killed (cytotoxicity) and the fraction of the surviving cells, in excess of those mutated by natural background, mutated at different concentrations (doses) of the material being tested. One measure of specific mutagenic activity useful for comparison purposes is the fraction, F, above the 95% background level, of surviving cells mutated at some constant dose. The larger the value of F, the greater the specific mutagenic activity of the sample. Here, using dose-effect curves that generally involved testing at doses of 30, 100, and sometimes 300 μg/mL, the quantity F was determined at a dose of 50 μg/mL. In this manner, F values were obtained for the material collected in each of the DCM contacting vessels, and for the combined DCM extracts from the filter, sorbent, and condensate.

It is also instructive to compare the amount of mutagenicity, M, estimated to be emitted by the oil burner, using the data provided by each sampling train. The total emitted mutagenicity accounts for the specific mutagenic activity of a given sample and the total amount of that sample emitted per weight of fuel combusted. The quantity M was computed from the information obtained with each sampler as follows. The corresponding quantity F was divided by the test dose (50 μg/mL), multiplied by the corresponding weight of sample collected, corrected for the fraction of oil burner exhaust educted through the sampling train, and normalized to a basis of unit weight of fuel fired, here 100 kg. The resulting calculated quantity can be interpreted as an estimate of the number of mutated bacterial cells that would be obtained if all the exhaust from burning 100 kg of fuel in the oil burner were directed at a suitable flow rate, see below) through the given sampling train, and all the extractables thus collected were tested in doses of 50 μg/mL. The larger the quantity M, the greater the number of emitted mutants detected by the given sampler.

Table 4 presents results on specific and total mutagenicity from three different experiments. The data show that the DCM sampler collected more material and more mutagens (higher F values), than did the filter/sorbent bed sampling train. Because of these two effects, estimates of total emissions of bacterial mutagens (M values) are significantly higher (factors of 3 to 20) when based on data from the DCM sampler.

Table 3. Yields of oil burner effluent extractables from DCM and filter/sorbent bed samplers.[a]

| Nominal excess air % | Mean sampling rate, SCFM | | No. of DCM baths[b] | Calulated average emitted yields of DCM extractables, mg/kg fuel | |
	DCM	Filter/sorbent		DCM sampler	Filter/sorbent sampler[c]
75	1.5	1.5	1	17 (1)[d]	58 (1)
70–85	0.84	0.89	2	319 (3)	69 (3)
66	0.95	—[e]	3	888 (1)	—[e]
62–75	0.42	0.48	4[f]	995 (3)	234 (2)

[a]Burner operating conditions: cyclic firing (5 min on, 10 min off). at Bacharach Smoke No. 1.
[b]Approximate bath temperatures: 1 −30°C; 2 through 4 (if used): −70°C.
[c]Includes DCM extract of filter, sorbent resin, and water from knockout vessel.
[d]Number of experiments in parentheses.
[e]Data not obtained.
[f]Glass beads added to baths 1 and 2.

Table 4. Specific and total mutagenicity of DCM solubles, inferred from side-by-side sampling with two different samplers.[a]

| Nominal excess air, % | Sampling rate, SCFM[b] | | Total weight of material collected, mg | | | | | | Specific mutagenic activity, F[d] | | | | | Calculated estimate of total mutagenicity, M[e] | | | | | |
| | DCM | F/S/W[c] | DCM vessel no. | | | | 1–4 | F/S/W | DCM vessel no. | | | | F/S/W | DCM vessel no. | | | | 1–4 | F/S/W |
			1	2	3	4			1	2	3	4		1	2	3	4		
75	0.40	0.47	6.6	14.69	12.02	13.11	46.42	19.49	29	25	27	9	17	45	86	76	28	235	65
75	0.49	0.51	5.52	31.55	16.89	20.64	74.60	22.16	6	4	6	6	2	6.3	24	19	23	72	8.1
62	0.42	0.44	10.37	30.75	14.42	22.37	77.91	22.37	17	3	4	9	1	44	23	14	50	131	5.3

[a]Cyclic (5 min on, 10 min off) firing of residential oil burner. See text.
[b]SCFM, standard cubic feet per minute.
[c]Filter/sorbent bed sampling train.
[d]F, 10^5 × mutant fraction at 50 μg/mL.
[e]M, bacterial cells mutated per 100 kg fuel fired. See discussion in text. Mutagenicity is to *S. typhimuium*, without exogenous metabolizing agents and was measured using the protocol of Skopek et al. (25,26). Higher values imply greater total mutagenicity.

Detection of High Volatile Organic Compounds in Atmospheric Pressure Combustion

Lafleur et al. (27) identified several highly volatile unsaturates including aliphatic poly-ynes, and mono- as well as poly-ene- and poly-yne-substituted benzenes in extracts obtained with the DCM sampler from an atmospheric pressure, turbulent premixed ethylene-air flame. Pentadiyne, 1-hexen-3,5-diyne, *m*- and *p*-diethynyl-benzene, and 1,3,5-hexatriyne were among the specific compounds detected. Compounds of this type are of great interest in determining the detailed chemistry of PAH formation at high temperatures (28). Further, some are suspect toxicants. A conventional filter/polymeric sorbent bed sampler designed for higher molcular weight PAHs may not detect these compounds because of their high volatility and chemical reactivity.

Operating Limits and Design Considerations for Other Applications

The performance features described were demonstrated for DCM collection vessels sized and configured as in Figures 1 and 2 and operated under narrowly defined conditions. When other vessel sizes and operating conditions are of interest, it is recommended that the design specifications and performance limitations described in the following paragraphs be carefully considered. Furthermore, in samplers of any size, solvent impurities may preclude reliable sampling of gaseous media with toxicant

concentrations below a critical value. Procedures for estimating minimum acceptable toxicants concentrations are also discussed.

Detailed mathematical analysis of sampler design and operation was outside the present scope, although modern separations science provides excellent resources for such an endeavor (29,30). However, a global mathematical representation of sampler performance is useful in discussing criteria for reliable sampler performance. For example, under steady-state sampling conditions, i.e., for constant sampling rate, collection efficiency, and inlet concentration of sample, the total yield of sample in a DCM collection train, Y, can be approximately estimated from the relation:

$$Y = n(S)SC_i t_s \qquad (1)$$

where S is the sampling rate in volume of gas per unit time, C_i is the concentration (in mass per unit volume) in the sampled stream at the inlet to the first collection vessel of material to be collected, and t_s is the total time of sampling. The quantity $n(S)$ is a sampling train efficiency, here defined as the fraction of sample entering the collection train that is retained within the DCM reservoir(s). Ideally, $n(S)$ would be unity and under certain conditions can be made to approach this limit. In general, however, $n(S)$ is < 1, and may depend on sampling rate, chemical reactions, size, geometry, and temperature of the collection vessel;

the fluid mechanics of sample stream-solvent contacting; and the concentrations (rigorously the chemical potentials) of sample in the sampled stream and in the solvent (29,30). These complexities, as well as the fact that in general $n(S)$, S, and C_i may each be time dependent, and ignored in the present, approximate treatment, but would require careful scrutiny in formal mathematical modeling for sampler design, operation, and automatic control.

Effects on sampler design and performance of the other quantities on the right-hand side of Eq. (1) are now considered.

Sampling Rate

Low sampling rates will furnish inadequate sample yields in reasonable collection times, while excessive rates will produce intolerable solvent and sample losses (and potentially, frit plugging by icing). Table 2 shows that for sampling times of about 60 to 120 min, a sampling train with three series-connected DCM impinger vessels provides high sampling efficiencies, i.e., minimal extractables breakthrough beyond collector 3, at sampling rates of about one-third SCFM (1.57×10^{-4} m^3/s). At higher flow rates, the DCM sampler still yields more extractables (Tables 3 and 4) and mutagens (Table 4) than does the filter/sorbent bed sampler, but breakthrough of product to the downstream collectors (Table 2 and 4) is excessive. Thus, sampling rates above about one-third SCFM cannot be recommended for quantitative work with samplers sized and configured as shown in Figures 1 and 2, respectively. Acceptable sampling rates, and corresponding sampling efficiencies, $n(S)$, should be experimentally determined for the specific collector vessel size and geometry, collection times, and gaseous media of interest.

Total Sampling Time

At constant values of C_i, S, and $n(s)$, Eq. (1) implies that cumulative sample yield should increase linearly with total sampling time. For $n(s)$ to be constant, the sample concentration in each DCM reservoir should be small compared to the maximum sample solubility in DCM at that DCM temperature. Excessive sampling times will obviously invalidate this condition and also cause intolerable carry over and loss of DCM. At sampling rates of about one-third SCFM and the DCM reservoir temperatures given earlier, experience shows that the present design accommodates sampling times of 1 to 7 hr with minimal solvent carry over (~ 10–15%).

Toxicants Concentration in the Media To Be Sampled

Even ultra-high purity DCM can contain small levels of impurities. Residue concentrations stated by suppliers are about 2 to 3 ppm (w/w), but measurements in this laboratory found DCM backgrounds of 0.25 to 1 ppm to be more typical. When toxicants identification, characterization, or quantitation is an objective of sampling, effects of solvent contaminants must be considered. The obvious strategy of further purifying the solvent prior to sampling is not easily applied here. Solvent decontamination into the tens of parts per billion range would be necessary for some environmental health sampling measurements now of interest. Without expensive clean room facilities and labor-intensive cleanup protocols, standard methods for liquids purification,

such as treatment on adsorption columns or hard-cut distillation, will not meet this level of cleanup and may result in new impurities and greater overall contamination of the solvent.

Assuming no chemical reactions between the solvent impurities and the sample, an alternative strategy is to operate so that the cumulative weight of sample collected, Y, substantially exceeds the total weight of DCM contaminants:

$$Y \gg r p_s V_s \tag{2}$$

where r is the concentration of the solvent impurities in weight per weight of solvent, p_s is the density of the solvent, and V_s is the volume of solvent in the reservoir containing Y. Assuming the validity of Eq. (1) and using it to substitute for Y gives

$$n(S)SC_i t_s \gg r p_s V_s \tag{3}$$

Thus, in principle, effects of solvent contaminants can be countered by sampling at high rates (increasing S) or for longer times (increasing t_s) and by employing a smaller DCM impinger vessel (decreasing V_s). However, as discussed ealier, for a fixed sampler size and geometry, sampling rate and sampling time may already be prescribed by other design or operating constraints. Normally, collection efficiency [$n(S)$] is already at a high level, leaving little elasticity to help increase Y. Furthermore, excessive reductions in V_s will degrade the strong organics dissolution, cooling, and dilution powers of the sampler. Thus, for many applications, Eqs. (2) and (3) reduce to a requirement that the inlet concentration of sample in the sampled stream C_i must exceed a minimum critical value, C_c:

$$C_i \gg r p_s V_s / n(S)St_s = C_c \tag{4}$$

A calculation illustrates the use of Eq. (4). Two 4-L DCM contacting vessels would utilize a total of 4 L of DCM. Assuming a DCM contamination level of 0.25 ppm (w/w), a collection efficiency, sampling rate, and sampling time of 1, one-third SCFM, and 120 min, respectively, gives:

$$\begin{aligned}
C_c &= [0.25 \times 10^{-3} \text{ mg/g DCM} \times 1.33 \text{ g/cm}^3 \\
&\quad \times 4000 \text{ cm}^3 \text{ DCM}]/ \times [1.0 \times 1/3 \text{ ft}^3/\text{min} \\
&\quad \times 120 \text{ min}] \\
&= 3.33 \times 10^{-2} \text{ mg/ft}^3
\end{aligned} \tag{5}$$

showing that in this case, if $C_i = 100 \times C_c = 3.33$ mg/ft^3, DCM background effects would impose an error of about 1% on the total yield determination. Extractables concentrations exceeding this value were observed in samples recovered with the DCM sampler from in-flame and postflame sampling of turbulent, premixed ethylene-air combustion. However, ambient and indoor air concentrations of extractables are typically much lower than 3.33 mg/ft^3. Further steps would therefore be required for reliable sampling of these atmospheres with a DCM sampler. In light of Eq. (3), options include reoptimizing the contactor vessel

size and operating conditions to retain desired performance features while accommodating smaller solvent volumes, larger sampling rates, and longer collection times.

Toxicological activity in DCM impurities may impose additional design or operating constraints in sampling gaseous media with dilute toxicant concentrations. For example, bacterial mutagenicity was detected in some residues from fresh DCM. The sample yield must therefore be so large that this background mutagenicity is negligible compared to the mutagenicity of the sample. Calculations of the required sampling conditions would, in general, require knowledge of the dose-response curves for the DCM residue and for the sample. Since the latter is normally one of the objectives of sampling, iterative estimation techniques would be necessary. Here a simplified case is considered to illustrate how the design calculation would be performed. To avoid complicated algebra, zero threshhold, linear dose response curves are assumed for the DCM impurity and for the sample. The quantities MF_r and MF_s are, respectively, the mutant fractions caused by the the DCM residue and the sample, i.e., the mutant fractions in excess of the 95% background mutation fraction. These can be written as

$$MF_r = A_r D_r \qquad (6)$$
$$MF_s = A_s D_s \qquad (7)$$

where subscripts r and s denote DCM residue and sample, respectively, A is the slope of the dose-response curve (in mutant fraction/dose), and D is the dose (in mass/unit volume of test medium). (For orientation purposes, the quantity F introduced in the discussion of Table 4 above is the value of MF_s at a dose of 50 $\mu g/mL$.) The condition for negligible contributions from the DCM background mutagenicity is

$$MF_s \gg MF_r \qquad (8)$$

i.e.,

$$A_s D_s \gg A_r D_r \qquad (9)$$

or

$$D_s / D_r \gg A_r / A_s \qquad (10)$$

Now D_s / D_r is directly proportional to the ratio of cumulative sample yield to total amount of DCM impurity:

$$D_s / D_r = Y / r p_s V_s \qquad (11)$$

Eq. (10) thus requires that

$$Y \gg (A_r / A_s) r p_s V_s \qquad (12)$$

Again assuming that Eq. (1) can be used to substitute for Y in Eq. (11) gives

$$n(S) SC_i t_s \gg (A_r / A_s) r p_s V_s \qquad (13)$$

or, expressing the criterion in terms of a lower bound on acceptable toxicants concentrations in the sampled medium,

$$C_i \gg (A_r / A_s) [r p_s V_s / n(S) St_s] \qquad (14)$$

The term in square brackets in Eq. (14) is the quantity C_c in

troduced in Eq. (4). Eq. (14) shows that if the DCM impurities exhibit specific mutagenic activity exceeding that of the sample (i.e., if $A_r > A_s$), the minimum sample concentration necessary for acceptable sampler performance Eq. (4) must be further increased by the factor (A_r / A_s).

Discussion: Implications for Environmental Health Sciences

Complex mixtures of wide boiling-range organic compounds are ubiquitous environmental contaminants. Assessment of their potential public health impacts requires knowledge of their composition, local concentrations, toxicological activity, sources, emissions factors, and formation mechanisms. For products of combustion and related high temperature processes such as incineration, this information must usually be obtained by off-line chemical analysis and toxicological testing of complex organic mixtures recovered from flames, exhausts, and other elevated temperature flows, and from ambient and indoor atmospheres. Reliable sampling apparatus is clearly essential to collecting representative, uncontaminated samples in sufficient quantities for in-depth characterization.

The DCM sampler is very well suited for combustion sampling. In sampling combustion exhaust side-by-side with a filter/polymeric sorbent bed sampler, the DCM equipment provided higher recoveries of S. typhimurium mutagens active without exogenous metabolizing agents and of total DCM solubles identified with complex organic mixtures. The DCM sampler exhibits high recovery and retention efficiencies for individual PAH compounds and for whole complex mixtures of DCM solubles. Minimal breakthrough of extractables beyond a second DCM contacting vessel (< 5%; Table 2) was detected in sampling a high throughput, turbulent premixed flame. This implies that a two-collector DCM sampling train, sized and configured as in Figures 1 and 2, respectively, should provide high, absolute collection efficiencies (>90%) when sampling continuous combustors within the prescribed sampling rates and total sampling times (~1/3 SCFM and ≤ 120 min). In general, however, DCM sampler performance, including collection efficiencies for whole mixtures, individual organic compounds, and various toxicants, will depend on collection vessel size and geometry, solvent type, temperature and impurities, sampling rate, sampling time, and sample concentration in the medium being interrogated. Limitations for the equipment shown in Figures 1 and 2 and general approaches to design and sizing for other applications are discussed above.

A test under conditions pertinent to combustion sampling resulted in < 1 part in 10^7 artifactual nitration of pure aromatic compounds dissolved in DCM. However, because of the very high bacterial mutagenicity of some nitroaromatics and the wide range of sampling conditions of environmental interest, nitration and all artifacts must still be scrutinized when employing the DCM sampler. For nitration artifacts, one approach to this end would be to employ in situ nitration detectors as discussed above.

The performances of the present DCM sampler and of a filter/polymeric sorbent bed sampler were compared in side-by-side sampling of residential oil burner effluents. The results (Tables 3 and 4) suggest that our earlier study of residential oil burner effluents using a filter/sorbent bed sampler (1,2) may have

underestimated total yields of organic extractables and possibly of some toxicants. This does not mean that the results from Learty et al. (*1*) and Braun et al. (*2*) or from other sampling studies based on filter/sorbent bed samplers are invalid, but rather that, if sampler-specific collection efficiencies are unavailable for the operating conditions employed, the observed yields of total organic extractables may constitute lower bounds on their actual emissions.

The direct dissolution, dilution, and cooling features of the DCM sampler are especially advantageous in recovering high yields of volatile, polar, and reactive organic compounds. Lafleur et al. (*27*) identified phenol, benzaldehyde, pentadiyne, 1-hexen-3,5-diyne, *m*- and p-diethynyl-benzene, 1,3,5,-hexatriyne, and other high volatile, low molecular weight ($<$ 126 amu) olefinic- and acetylenic-substituted benzenes, in complex mixtures obtained with this sampler from an atmospheric pressure, turbulent premixed ethylene-air flame. These compounds were obtained in the same master sample that contained high molecular weight compounds including PAH of up to at least five rings. No special effort was made to preferentially sample for low boiling-point compounds. Data on the identities and concentrations of volatile and reactive organic compounds in flames are important in determining the chemistry of PAH and soot formation and depletion, and thus in understanding how combustion generates bioactive effluents. For example, Bittner and Howard (*28*) detected polyacetylenes in low pressure ($\sim$ 20 torr) laminar premixed benzene-oxygen-argon flames using on-line molecular beam mass spectrometry and assigned acetylene and diacetylene important mechanistic roles in hydrocarbon decay.

Little seems to be known about the bioactivity of volatile organic unsaturates of the types recovered with the DCM sampler. However, the well-established carcinogenicity of at least one conjugated aliphatic diene, butadiene, suggests that aromatics with unsaturated aliphatic substituents, and aliphatic unsaturates themselves, warrant toxocological characterization.

The DCM sampler provides large quantities ($\sim$ 100–250 mg) of DCM extractables by sampling flames or their effluents for relatively short times. This sampler may thus be able to supply traditionally unavailable organics in quantities sufficient for use as standards in chemical analysis and for detailed toxicological testing of individual compounds.

Polar organic compounds have been associated with bacterial cell mutagenicity in wood smoke (*31*) and residential oil burner effluents (*1,2,32*) and command increasing attention as combustion-generated toxicants. Because of their high reactivity, low boiling points, and (for polar compounds) poor affinity for certain sorbents, polar or unsaturated organic volatiles may be harder to detect and quantify with sampling trains using a filter and single type of sorbent when simultaneous collection of less volatile polycyclic organics is also desired. A gas sampling loop should recover the more volatile of these compounds, but in small yields, making workup, chemical analysis, and toxicological characterization more difficult. Furthermore, separate, upstream collectors would typically be required for simultaneous collection of less volatile organics. The DCM sampler simplifies combustion sampling of organics of wide-ranging volatility and chemical functionality by providing, in a single collection vessel, high yields of organic compounds

spanning broad ranges of boiling point, polarity, and reactivity.

By employing proper design and operating criteria, including characterization of collection efficiencies and artifacts generation behavior for the conditions of interest, the above or appropriately modified versions of the DCM sampler are expected to be applicable to toxicological assessment of combustors, incinerators, and other high-temperature process streams and of ambient air sheds and indoor atmospheres. The sampler is expected to contribute to public health impact assessments by facilitating detailed determinations of the identities, compositions, concentrations, sources, formation mechanisms, and biological activity of environmental toxicants.

REFERENCES

1. Leary, J. A., Bieman, K., Lafleur, A. L., Kruzel, E. L., Prado, G. P., Longwell, J. P., and Peters, W. A. Chemical and toxicological characterization of residential oil burner emissions: I. Yields and chemical characterization of extractables from combustion of no. 2 fuel oil at different Bacharach smoke numbers and firing cycles. Environ. Health Perspect. 73: 223–234 (1987).
2. Braun, A. G., Busby, Jr., W. F., Liber, H. L., and Thilly, W. G. Chemical and toxicological characterization of residential oil burner emissions: II. Mutagenic, tumorigenic, and potential teratogenic activity. Environ. Health Perspect. 73: 235–246 (1987).
3. Howard, J. B., and Longwell, J. P. Formation mechanisms of PAH and soot in flames. In: Polynuclear Aromatic Hydrocarbons: Formation, Metabolism, and Measurement (M. Cooke and A. J. Dennis, Eds.), Battelle Press, Columbus, OH, 1983, pp. 27–62.
4. Liber, H. L., Andon, B. M., Hites, R. A., and Thilly, W. G. Diesel soot: mutation measurements in bacterial and human cells. Environ. Int. 5: 281–284 (1982).
5. Barfknecht, T. R., Hites, R. A., Cavaliers, E. L., and Thilly, W. G. Human cell mutagenicity of polycyclic aromatic hydrocarbon components of diesel emissions. In: Toxicological Effects of Emissions from Diesel Engines (J. Lewtas, Ed.), Elsevier Press, New York, 1982, pp. 277–294.
6. Busby, Jr., W. F., Stevens, E. K., Kellenbach, E. R., Cornelisse, J., and Lugtenburg, J. Dose-response relationships of the tumorgenicity of cyclopenta(cd)pyrene, benzo(a)pyrene and 6-nitrochrysene in a newborn mouse lung adenoma bioassay. Carcinogenesis 9: 741–746 (1988).
7. Pitts, J. N., Van Cauwenberghe, K. A., Grosjean, D., Schmid, J. P., Fitz, D. R., Belser, W. L., Knudson, G. B., and Hynds, P. M. Atmosphere reactions of polycyclic aromatic hydrocarbons: facile formation of mutagenic nitro derivatives. Science 202: 515–519 (1978).
8. Houk, V. S., Zweidinger, R. B., and Claxton, L. D. Mutagenicity of Teflon-coated glass fiber filters: a potential problem and solutions. Environ. Sci. Technol. 21: 917–920 (1987).
9. Alfheim, I., and Lindskog, A. Sci. Total Environ. 34: 203–222 (1984).
10. Pitts, J. N. Atmospheric interactions of nitrogen oxide. In: Nitrogen Oxides and Their Effects on Health (S. D. Lee, Ed.), Ann Arbor/Butterworth Group, Woburn, MA, 1980, p. 88.
11. Tokiwa, H., Nakagawa, R., Morita, K., and Ohnishi, Y. Mutagenicity of nitro derivatives induced by exposure of aromatic compounds to nitrogen dioxide. Mutat. Res. 85: 195–205 (1981).
12. Stenburg, R. L., von Lehmden, D. J., and Hangebrauck, R. P. Sample collection techniques for combustion sources — benzopyrene determination. Am. Ind. Hyg. Assoc. J. 22: 271–275 (1961).
13. Pitts, Jr., J. N. Atmospheric interactions of nitrogen oxides. In: Nitrogen Oxides and Their Effects on Health (S. D. Lee, Ed.), Ann Arbor Science, Ann Arbor, MI, 1980, pp. 77–93.

14. O'Brien, R. J., Green, P. J., Doty, R. A., Vanderzanden, J. W., Easton, R. R., and Irwin, R. P. Interaction of oxides of nitrogen and aromatic hydrocarbons under simulated atmospheric conditions. In: Nitrogenous Air Pollutants, Chemical and Biological Implications (D. Grosjean, Ed.), Ann Arbor Science, Ann Arbor, MI, 1980, pp. 189–210.

15. Chan, T. L., and Gibson, T. L. Sampling and atmospheric chemistry of particles containing nitrated polycyclic aromatic hydrocarbons. In: Nitrated Polycyclic Aromatic Hydrocarbons (C. M. White, Ed.), Huethig, Heidelberg, 1985, pp. 237–264.

16. Pryor, W. A. Mechanisms and detection of pathology caused by free radicals, tobacco smoke, nitrogen dioxide and ozone. In: Environmental Health Chemistry (J. D. McKinney, Ed.), Ann Arbor Science, Ann Arbor, MI, 1981, pp. 445–466.

17. Tokiwa, H., Nakagawa, R., Morita, K., and Ohnishi, Y. Mutagenicity of nitro derivatives induced by exposure of aromatic compounds to nitrogen dioxide. Mutat. Res. 85: 195–205 (1981).

18. Lafleur, A. L., and Mills, K. M. Trace level determination of selected nitroaromatic compounds by gas chromatography with pyrolysis-chemiluminescent detection. Anal. Chem. 53: 1202–1205 (1981).

19. Phillips, J. H., Coraor, R. J., and Prescott, S. R. Determination of nitroaromatics in biosludges with a gas chromatograph/thermal energy analyzer. Anal. Chem. 55: 889–892 (1983).

20. Tomkins, B. A. Chromatographic detectors used for the determination of nitrated polycyclic aromatic hydrocarbons. In: Nitrated Polycyclic Aromatic Hydrocarbons (C. M. White, Ed.), Huethig, Heidelberg, 1985, pp. 87–120.

21. Yu, W. C., Fine, D. H., Chiu, K. S., and Biemann, K., Determination of nitrated polycyclic aromatic hydrocarbons in diesel particulates by gas chromatography with chemiluminescent detection. Anal. Chem. 56: 1158–1162 (1984).

22. Rosenkranz, H. S., and Mermelstein, R. Mutagenicity and genotoxicity of nitroarenes: all nitro-containing chemicals were not created equal. Mutat. Res. 114: 217–267 (1983).

23. Nishioka, M. G., Petersen, B., and Lewtas, J. Comparison of nitro-aromatic content and direct acting mutagenicity of passenger car engine emissions. In: Mobile Source Emissions Including Polycyclic Organic Species (D. Rondia, M. Cooke, and R. K. Haroz, Eds.), D. Reidel Publishing Co., Dordrecht, The Netherlands, 1983, pp. 197–210.

24. Nenniger, J. E. Polycyclic Aromatic Hydrocarbons Production in a Jet Stirred Combustor. ScD Thesis, Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA, 1983.

25. Skopek, T. R., Liber, H. L., Krolewski, J. J., and Thilly, W. G. Quantitative Forward mutation assay in *Salmonella typhimurium* using 8-azaguanine resistance as a genetic marker. Proc. Natl. Acad. Sci. USA 75: 410–414 (1978).

26. Skopek, T. R., Liber, H. L., Kaden, D. A., and Thilly, W. G. Relative sensitivities of forward and reverse mutation assays in *Salmonella typhimurium*. Proc. Natl. Acad. Sci. USA 75: 4465–4469 (1978).

27. Lafleur, A. L., Gagel, J. J., Longwell, J. P. and Monchamp, P. A., Identification of aromatic alkynes and acyclic polyunsaturated hydrocarbons in the output of a jet-stirred combustor. Energy Fuels 2: 709–716 (1988).

28. Bittner, J. D., and Howard, J. B. Mechanism of hydrocarbon decay in fuel-rich secondary reaction zones. In: Proceedings, 19th Symposium (International) on Combustion, The Combustion Institute, Pittsburgh, PA, 1983, pp. 211–221.

29. Cussler, E. M. Diffusion, Mass Transfer in Fluid Systems. Cambridge University Press, New York, 1984.

30. Perry, R. H., and Green, D. Perry's Chemical Engineers' Handbook, Sixth Edition. McGraw-Hill, New York, 1984.

31. Alfheim, I., Becher, G., Hongslo, J. K., and Ramdahl, T. Mutagenicity testing of high performance liquid chromatography fractions from wood stove emissions samples using a modified *Salmonella* assay requiring smaller sample volumes. Environ. Mutagen. 6: 91–102 (1984).

32. Leary, J. A., Lafleur, A. L., Liber, H. L., and Biemann, K. Chemical and toxicologic characterization of fossil fuel combustion product phenalenone. Anal. Chem. 55: 758–761 (1983).